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Hepatitis B Virus Genotype C Takes a More Aggressive Disease Course Than Hepatitis B Virus Genotype B in Hepatitis B e Antigen-Positive Patients

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One hundred forty-six hepatitis B e antigen (HBeAg)-positive chronic hepatitis B patients were followed up for 32 ± 13 months. All six patients with hepatocellular carcinoma had hepatitis B virus (HBV) genotype C. Disease activity was greater in patients infected by HBV genotype C than in those infected by HBV genotype B in the HBeAg-positive phase but not after HBeAg seroconversion.

Previous studies in Southeast Asia suggest that hepatitis B virus (HBV) genotype C is associated with a higher prevalence of hepatitis B e antigen (HBeAg) (6, 12, 14), more active hepatitis (3, 8, 11, 13), more advanced liver disease (7, 10), and a higher prevalence of hepatocellular carcinoma (5, 10, 16) than is HBV genotype B. However, most of them are either cross-sectional or case-control studies. Here we investigated the HBV genotypes and disease activity in a prospective longitudinal cohort of HBeAg-positive patients in Hong Kong.

One hundred seventy-two consecutive chronic hepatitis B patients who were positive for HBeAg at the initial clinic visit from December 1997 to November 1998 were prospectively followed up at 6-month intervals (1). Patients who had a hepatitis C and hepatitis D coinfection, alcoholism, or previous antiviral treatment were excluded. Clinical liver cirrhosis was defined as ultrasonic liver cirrhosis plus hypersplenism, esophageal varices, and/or ascites. Active liver disease was defined as either (i) an abrupt elevation of the alanine transaminase (ALT) level (>200 IU/liter or a more-than-threefold increase from the baseline level, whichever was greater) or (ii) a modest elevation of the ALT level (<200 IU/liter and a less-than-threefold increase from the baseline level) accompanied by HBeAg positivity or detectable HBV DNA (13, 15).

Hepatitis B surface antigen, anti-hepatitis C virus, and anti-hepatitis D virus were tested by enzyme-linked immunosorbent assay (Abbott GmbH Diagnostika, Wiesbaden-Delkenheim, Germany). HBeAg and anti-HBe were measured by enzyme-linked immunosorbent assay (Sanofi Diagnostics, Pasteur, France). HBV DNA was measured by DNA cross-linking assay (NAXCOR XLnt; NAXCOR, Menlo Park, Calif.) (2, 9). HBV genotyping was performed as described previously (3, 11). In short, extracted HBV DNA was amplified by PCR with primers flanking the HBV genome between nucleotides 256 and 796 (sense primer, 5′-GTGGTGGACTTCTCTCAATTTTC; antisense primer, 5′-CGGTA[A/T]AAAGGGACTCA[A/C]GAT). The PCR product was then incubated with restriction enzymes *Tsp*5091 (New England BioLabs, Beverly, Mass.) and *Hin*fI (Boehringer Mannheim, Mannheim, Germany), respectively. The restriction pattern on an agarose gel was visualized by ethidium bromide staining.

Categorical data were compared by a two-tailed chi-square test or Fisher's exact test, as appropriate. Continuous data were compared by a two-tailed Student *t* test. Statistical significance was taken as $P < 0.05$.

One hundred forty-six patients, including 87 males and 59 females aged 33 ± 13 years, followed up for 32 ± 13 months were PCR positive and were included in our HBV genotype study (Fig. 1). Sixteen patients (11%) had clinical liver cirrhosis. Eight-six patients (59%) were persistently HBeAg positive for 28 ± 14 months during the follow-up period. Sixty patients (41%) underwent HBeAg seroconversion. Overall, HBV genotypes B and C were found in 41 (28%) and 105 (72%) of the patients, respectively. There was no difference in the age, gender ratio, ALT level, proportion of patients with liver cirrhosis, or follow-up duration between the patients infected by the two different HBV genotypes (Table 1).

Hepatocellular carcinoma was found in six patients (4%) aged (mean ± standard deviation) 52 ± 10 years at 12 ± 5 months after the initial visit. All of them were male, persistently HBeAg positive, and infected by HBV genotype C (versus genotype B, $P = 0.082$). Four patients who had hepatocellular carcinoma at initial presentation and were not analyzed in this study were also infected by HBV genotype C (Fig. 1). Eleven patients (11%) infected by HBV genotype C, versus five patients (12%) infected by HBV genotype B, had clinical liver cirrhosis ($P = 1.00$).

Excluding the six patients who developed hepatocellular carcinoma, 20 patients with HBV genotype B and 60 patients with HBV genotype C did not undergo HBeAg seroconversion during the follow-up (Table 2). Among them, a significantly greater proportion of patients infected by HBV genotype C than of those infected by HBV genotype B had active disease. Twenty-one (51%) of 41 patients with HBV genotype B and 39 (37%) of 105 patients with HBV genotype C underwent documented HBeAg seroconversion ($P = 0.17$) (Table 3). There was no difference in the age at seroconversion, the proportion of patients with active disease post-HBeAg seroconversion, or

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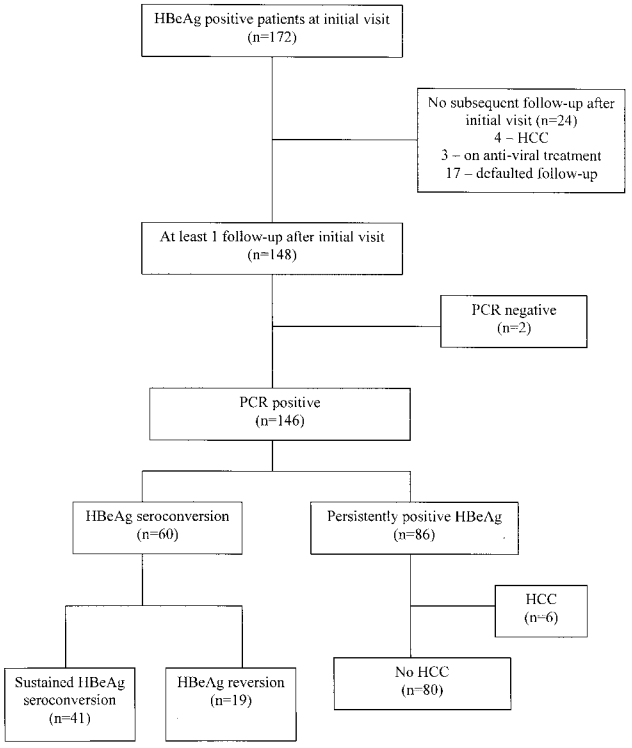

FIG. 1. Clinical status of chronic hepatitis B patients included in this study. HCC, hepatocellular carcinoma.

HBeAg reversion between patients infected by the two HBV genotypes.

The results of this study concur with previous studies indicating that HBV genotype C infection is associated with more aggressive disease (3, 8,10, 12). In this study, the ALT levels of patients infected by the two different HBV genotypes were comparable at the initial visit but the difference in disease activity became apparent on subsequent follow-up. This reflects the facts that some HBeAg-positive patients have fluctuating ALT levels and that disease activity cannot be accurately assessed by a snapshot laboratory result.

Previous studies have suggested that HBV genotype C is associated with a longer HBeAg positivity phase and delayed HBeAg seroconversion than is HBV genotype B (4, 10, 12, 14). However, this phenomenon was not confirmed by this study. As previous studies were either cross-sectional or retrospective, patient sampling and the high rate of PCR negativity, causing failure of HBV genotyping, might have biased their results (1, 4). In this study, as all patients were HBeAg positive at the initial visit, 99% of the serum samples were PCR positive for HBV genotyping. The prospective longitudinal follow-up of this study also allowed more accurate assessment of the timing of HBeAg seroconversion.

Data on the disease activity of HBeAg-negative patients infected by different HBV genotypes are conflicting (3, 15). The longitudinal study by Chu et al. suggested that a larger proportion of patients infected by HBV genotype C versus HBV genotype B have abnormal ALT levels after HBeAg seroconversion (4). In the present study, in which a combined ALT and HBV DNA criterion was used to define active liver disease, the difference between the two HBV genotypes in disease activity after HBeAg seroconversion did not reach statistical significance. In both studies, there was no difference in the rate of HBeAg reversion after seroconversion.

All patients who developed hepatocellular carcinoma in this study were infected by HBV genotype C. This echoes the findings of previous studies in Taiwan and Japan showing that hepatocellular carcinoma is predominantly found among patients infected by HBV genotype C (5, 10, 16).

In summary, the results of this study show that HBV genotype C is associated with more aggressive disease in the

TABLE 1. Baseline characteristics of patients infected by HBV genotypes B and C

Parameter	Genotype B (n = 41)	Genotype C (n = 105)	P value
Age (yr)	34 ± 13	33 ± 14	0.88
Male-female ratio	25:16	62:43	0.98
Initial ALT level (IU/liter)	83.5 ± 82.7	124.7 ± 162.4	0.083
Initial ALT level (no., %):			0.37
Normal	22, 54	45, 43	
1–2 × ULN[a]	11, 27	29, 28	
2–5 × ULN	7, 17	20, 19	
>5 × ULN	1, 2	11, 11	
Cirrhosis (no., %)	5, 12	11, 11	1.00
Follow-up period (mo)	32 ± 13	32 ± 13	0.99

[a] ULN, upper limit of normal.

TABLE 2. HBV genotypes and disease activity of patients who did not undergo HBeAg seroconversion[a]

Parameter	Genotype B (n = 20)	Genotype C (n = 60)	P value
Age (yr)	37 ± 16	32 ± 13	0.24
Male-female ratio	11:9	38:22	0.69
Follow-up period (mo)	25 ± 15	30 ± 14	0.24
Active disease (no., %)	10, 50	47, 78	0.032

[a] Not including six patients who developed hepatocellular carcinoma.

TABLE 3. HBV genotypes and disease activity of patients who underwent HBeAg seroconversion on follow-up

Parameter	Genotype B (n = 21)	Genotype C (n = 39)	P value
Age (yr) at HBeAg seroconversion	33 ± 9	34 ± 13	0.76
Male-female ratio	14:7	18:21	0.21
Follow-up period (mo)	39 ± 8	38 ± 8	0.74
Post-HBeAg seroconversion (no. of patients with >6-mo follow-up)	16	31	
Post-HBeAg seroconversion follow-up period (mo)	25 ± 10	20 ± 9	0.053
Active disease (no., %)	4, 25	16, 45	0.30
HBeAg reversion (no., %)	6, 38	13, 42	1.00

HBeAg-positive phase than is HBV genotype B. This may contribute to a higher risk of hepatocarcinogenesis. These findings are important in the clinical decision of HBV treatment, as well as hepatocellular carcinoma surveillance.

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